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TITLE: Epigenetic Silencing and Resistance to Imatinib Mesylate in CML

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CONTRACTING ORGANIZATION: University of Texas M.D. Anderson Cancer Center
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14. ABSTRACT

We performed analyses of DNA methylation data reported in the final report with clinical outcome of CML patients. DNA methylation significantly correlated with survival. CML patients with average methylation z-score values above zero had significantly shorter survival than patients with z-scores below zero (median 10 months vs 59 months, P<0.0001). This was true also within CP (median survival of 44 months for patients with high methylation vs median not reached in patients with low methylation, P=0.02) and AP (median survival of 11 months for patients with high methylation vs 27 months in patients with low methylation, P=0.009). Changes in gene silencing by DNA methylation may play a role in developing alternative routes for cells to circumvent the effects of imatinib.

15. SUBJECT TERMS
Chronic myelogenous leukemia, epigenetics, DNA methylation, decitabine, imatinib, clinical trial

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FINAL ADDENDUM REPORT
USAMRMC FY02 CHRONIC MYELOGENOUS LEUKEMIA RESEARCH PROGRAM

Grant/Contract/MIPR No.:	DAMD17-03-1-0448
Principal Investigator:	Jean-Pierre Issa, M.D.
Institution:	University of Texas M.D. Anderson Cancer Center Houston, Texas
Report Title:	Epigenetic Silencing and Resistance to Imatinib Mesylate in CML
Report Type:	Final Addendum
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We performed analyses of DNA methylation data reported in the final report with clinical outcome of CML patients. DNA methylation significantly correlated with survival. CML patients with average methylation z-score values above zero had significantly shorter survival than patients with z-scores below zero (median 10 months vs 59 months, $P<0.0001$). This was true also within CP (median survival of 44 months for patients with high methylation vs median not reached in patients with low methylation, $P=0.02$) and AP (median survival of 11 months for patients with high methylation vs 27 months in patients with low methylation, $P=0.009$). Changes in gene silencing by DNA methylation may play a role in developing alternative routes for cells to circumvent the effects of imatinib.

We expanded our analysis in CML patients to HOX developmental genes. The HOX gene family consists of 39 genes organized in 4 clusters A-D on 4 chromosomes in 13 paralogous groups. These genes regulate developmental processes and tissue differentiation. HOX gene translocations are frequently found in leukemia. Most HOX genes have CpG islands at their TSS. Silencing of HOX genes by DNA methylation may disrupt normal development of blood cells and thus be involved in leukemic transformation. We performed comprehensive DNA methylation analyses of 31 HOX genes with CpG islands in transcription start site (TSS) regions (HOXA1, 4, 5, 6, 7, 9, 10a, 10b, 11 and 13; HOXB1, 4, 5, 7, 8, 9, and 13; HOXC4, 5, 8, 9, 10, 11, and 13; HOXD1, 4, 8, 9, 10, 11, and 13). We analyzed DNA in blood cells from 20 CML

patients, comparing the results with 25 normal controls. We used bisulfite pyrosequencing to quantitate levels of cytosine methylation in CpG islands at TSS. Statistical analyses were performed with nonparametric tests. Asterisks (*) denote statistically significant methylation differences from normal controls ($p<0.05$). HOXA5 and HOXB1 genes showed dense methylation both in normal controls and in primary leukemic cells. HOXA4, A6 and HOXC4 genes showed moderately elevated methylation in normal controls (medians 13%, 15%, and 34%) and increased methylation in CML (87%*, 32%* and 28%). The remaining HOX genes were not methylated in normal controls. HOXB8 and B13 showed increased median methylation levels in CML (14% and 17%*). HOXC11 and C13 median methylation levels were also increased in CML (25%* and 13%). HOXD4 and D11 genes also showed a tendency to increased methylation in some CML patients (medians 17% and 25%).

Reportable outcomes

Poster presentation at the Annual Meetings of the American Society of Hematology 2006

Jelinek J, Gharibyan V, Oki Y, Chung W, Shen L, Cortes JE, Kantarjian HM, Issa JPJ. Aberrant DNA Methylation in CML Is Associated with Disease Progression and Resistance to Imatinib Mesylate. Blood 2006; 108: 622a

Poster presentation at Annual Meeting of the American Association for Cancer Research 2007

DNA methylation of HOX genes in leukemia and myeloproliferative disorders. American Association for Cancer Research Annual Meeting, Los Angeles, CA, April 14-18, 2007.

Oral presentation at the "Road to a Cure: the Chronic Myelogenous Research Program Investigators Meeting" Orlando, FL, December 7-8, 2006
DNA methylation in CML increases with disease progression, and associates with resistance to imatinib mesylate and shortened survival.

Figure 1. Increased DNA methylation average z-score is associated with shorter survival in CML patients. Low methylation, average z-score below 0; high methylation, average z-score above 0. CP, chronic phase; AP, accelerated phase; BC, blast crisis.

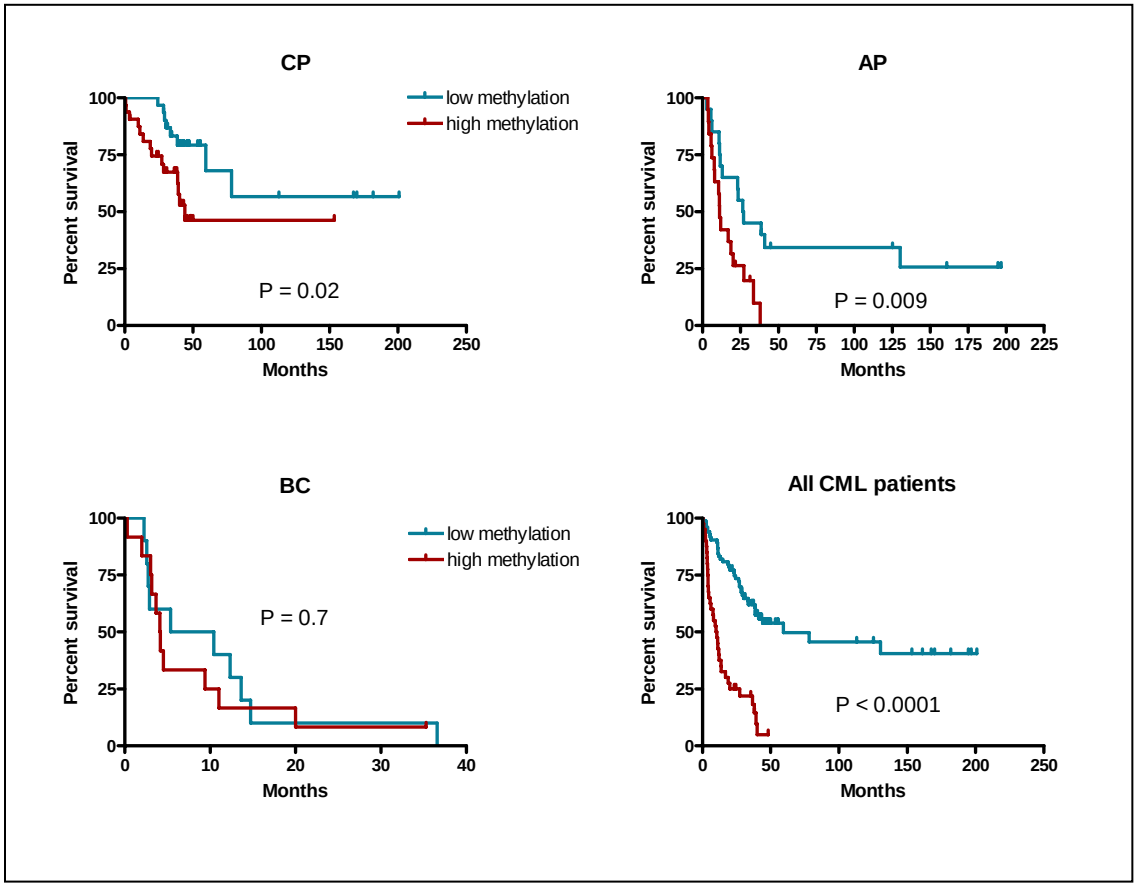
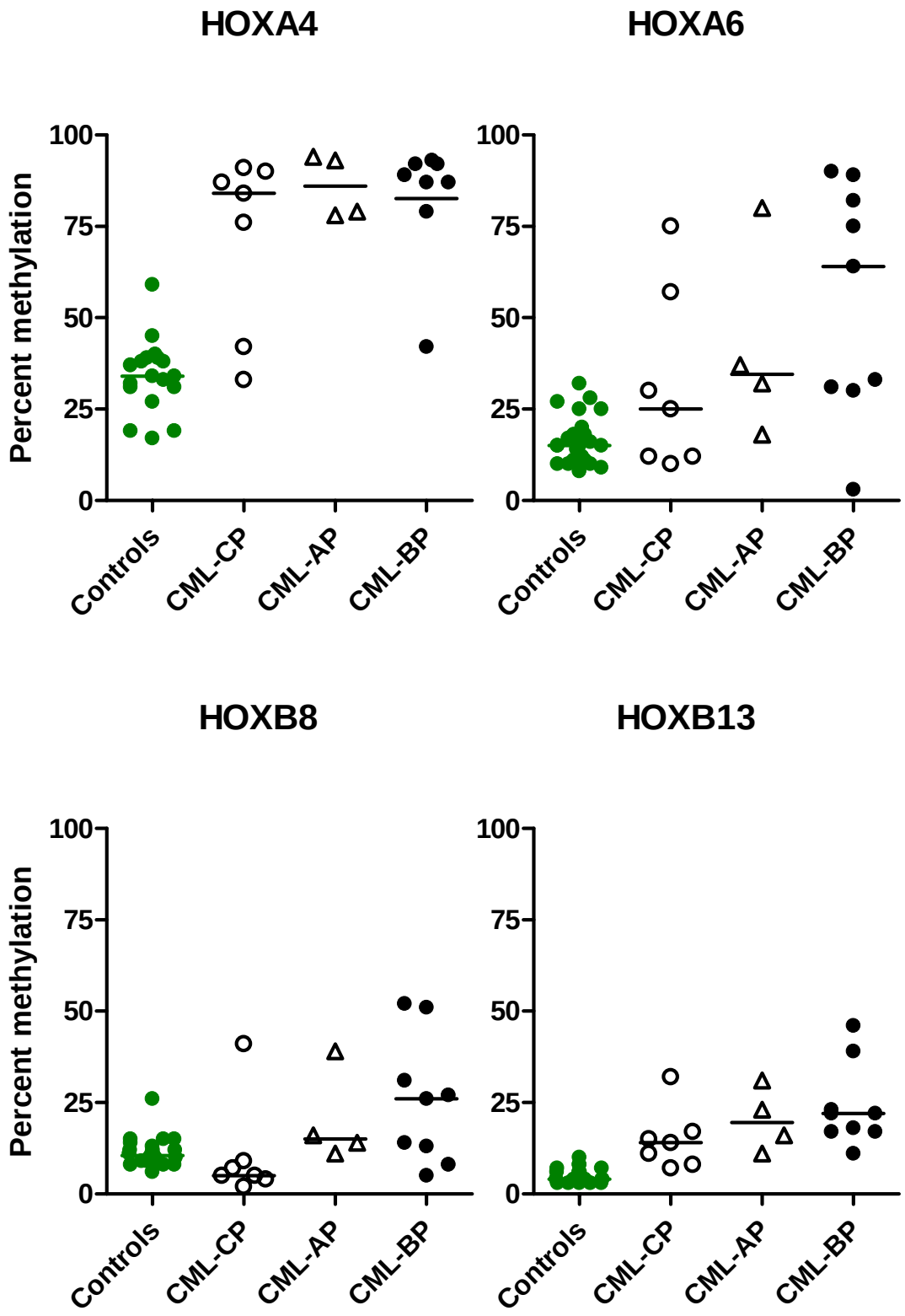
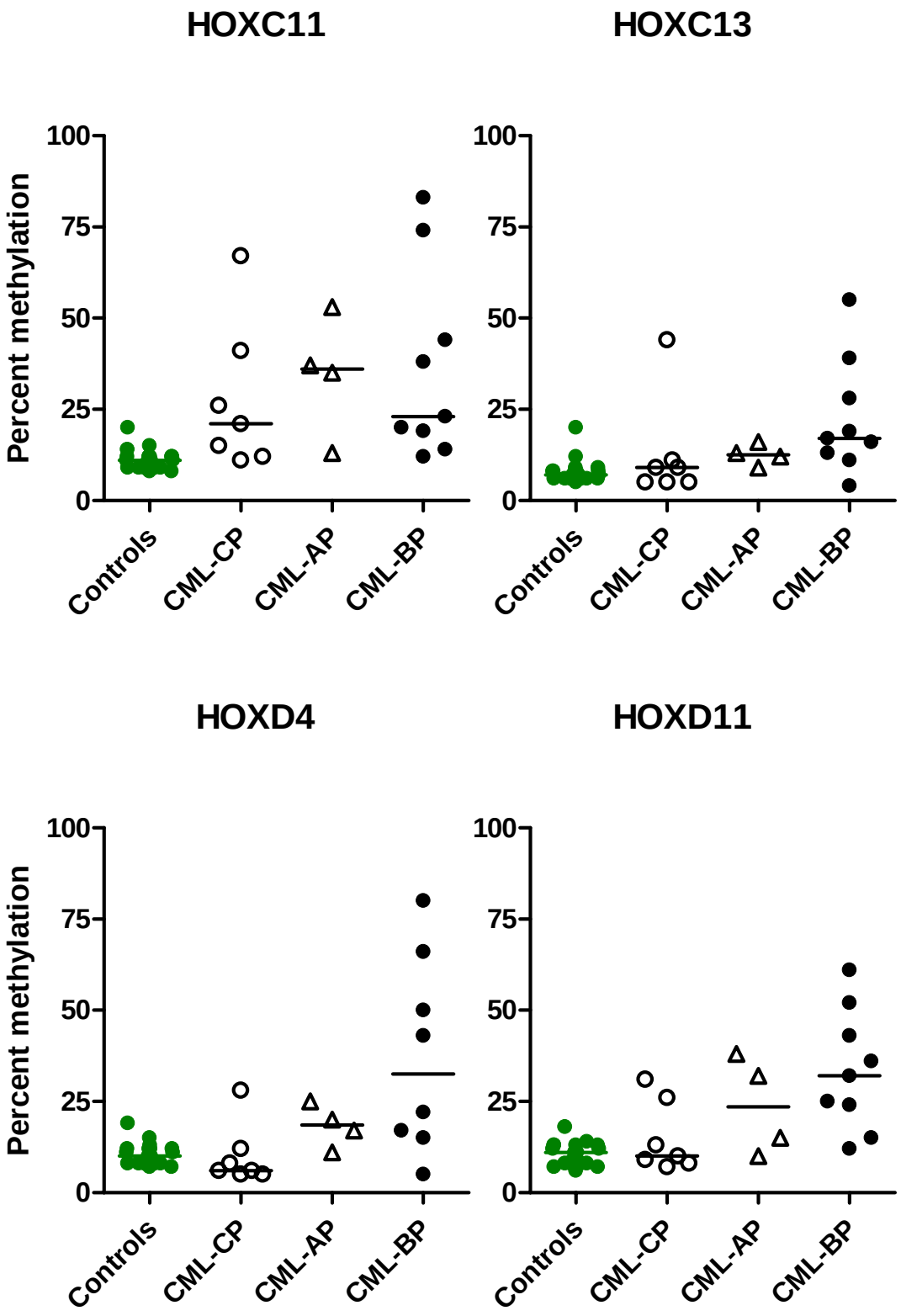


Figure 2. Methylation of HOX genes in CML patients and normal controls.




HOXC11
HOXC13
HOXD4
HOXD11
Percent methylation
100
75
50
25
0
Controls
CML-CP
CML-AP
CML-BP